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An automated, polymer-assisted strategy for the preparation of urea and thiourea derivatives of 15-membered azalides as potential antimalarial chemotherapeutics

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ABSTRACT

A series of 15-membered azalide urea and thiourea derivatives has been synthesized and evaluated for their in vitro antimalarial activity against chloroquine-sensitive (D6), chloroquine/pyremethamine resistant (W2) and multidrug resistant (TM91C235) strains of *Plasmodium falciparum*. We have developed an effective automated synthetic strategy for the rapid synthesis of urea/thiourea libraries of a macrolide scaffold. Compounds have been synthesized using a solution phase strategy with overall yields of 50–80%. Most of the synthesized compounds had inhibitory effects. The top 10 compounds were 30–65 times more potent than azithromycin, an azalide with antimalarial activity, against all three strains.
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1. Introduction

Today approximately 40% of the world's population is at risk of malaria, mostly those living in the poorest countries.[1] Malaria is found throughout the tropical and subtropical regions of the world and causes more than 300 million acute illnesses as well as 0.7–2.7 million deaths annually.[2,3]

In humans, malaria is caused by four strains of single-celled eukaryotic protozoa of the genus Plasmodium. These strains include *Plasmodium malariae*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium falciparum* of which *P. falciparum* is by far the most deadly.[4,5] *P. falciparum* causes malaria tropica, which without treatment, is often lethal for the infected patient.

The worldwide increase in drug-resistance to most affordable chemotherapies,[6,7] such as chloroquine **1**, has created a demand for the development of new malaria treatments. Currently, artemisinin **2** and its derivatives are the drugs of choice to treat multidrug resistant malaria[8] (Fig. 1).

With the recent introduction of artemisinin-based combination therapy as the first-line therapy in many countries where malaria is endemic, combination therapies have become the standard of care for the treatment of *P. falciparum* malaria. However, the spread of multidrug resistance from Southeast Asia to significant parts of the world where malaria is endemic, calls for the development of novel antimalarial compounds. The challenge today in malaria chemotherapy is to find safe, effective and affordable new drugs lacking cross-resistance with standard antimalarial drugs.[9]

Antibiotics with antimalarial activity may offer an interesting alternative for the treatment of multidrug resistant falciparum malaria. Azithromycin, a widely prescribed advanced-generation macrolide antibiotic with a favorable toxicological profile, has shown intrinsic activity against *Plasmodium* spp. both in vitro[10,11] and in vivo in prophylaxis and in treatment.[12–18] Compared to other antibiotics used for malaria treatment (e.g., tetracyclines), azithromycin offers unique advantages due to its safety in children and experience with use in pregnant subjects,[19] the populations most affected by malaria.

Through screening a set of in-house compounds from our compound bank against a panel of parasites, various urea- and thiourea derivatives **3** of azithromycin were found to have higher in vitro potency than azithromycin (Fig. 2). Therefore, our effort has been focused on the development of an automated synthetic strategy to quickly and efficiently produce urea and thiourea libraries of a

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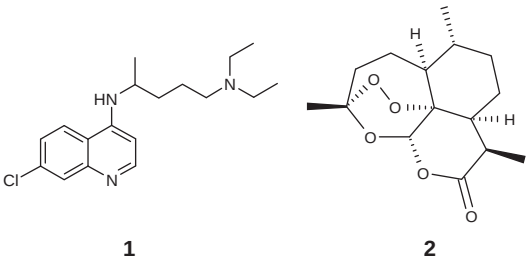


Figure 1. Chloroquine and artemisinin.

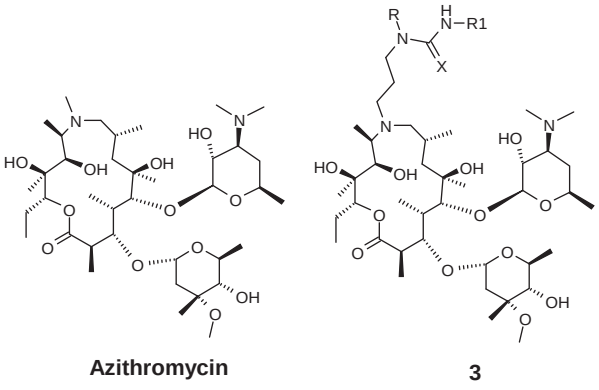


Figure 2. Azithromycin and potentially active macrolide derivatives.

macrolide scaffold for the screening of potential antimalarial compounds.

2. Results and discussion

2.1. Chemistry

The primary goal within any medicinal chemistry program is to rapidly identify a lead compound in order to initiate the synthesis of suitable derivatives and ultimately identify candidates with good ADME properties, efficacy and safety. Solid-phase synthesis techniques continue to be widely exploited for the preparation of large compound libraries.[20] This strategy has been further enhanced by the incorporation of automated compound synthesis and processing protocols.[21] However, in many cases, difficulty in monitoring solid-phase chemistry, the inability to purify resin-bound intermediates, and protracted chemistry development times have focused attention on the need to develop complementary solution phase strategies for high-throughput chemistry.[22]

It is important when planning automated syntheses that the synthetic procedures are chosen and optimized with regards to the capabilities and characteristics (tube sizes, reaction block formats, etc.) of the synthesizer to be used. Each step, such as solvent addition, reagent addition, and evaporation, is considered in terms of an automated synthesis because each step could represent a total success or failure in the preparation of libraries. For this reason, it is helpful to represent the synthesis in the form of a flowchart in which each successive synthesis operation is shown (Fig. 3). Moreover, it is preferable to rehearse chemistry on the robot from the outset in order to develop robust protocols in the shortest time.

2.1.1. Preparation of trial library compounds 7{1–6} and 8{1–6}

Our approach to the 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) urea derivatives **7**{1–6}, and the corresponding 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) thiourea derivatives

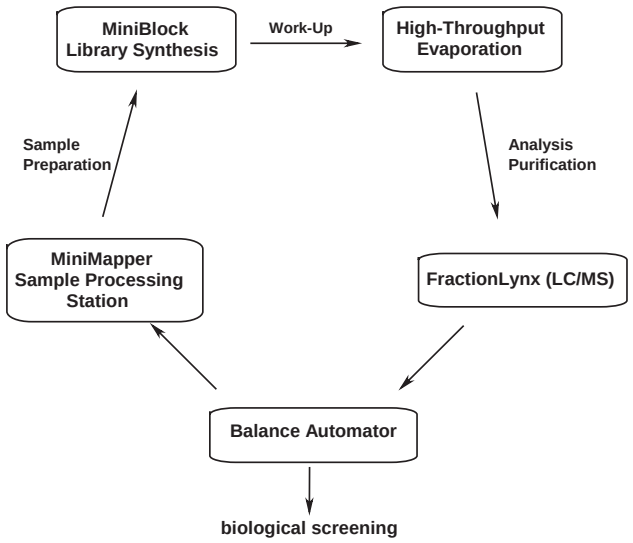


Figure 3. Automated 9a-aza-homoerythromycin-1-propyl urea and thiourea library synthesis flowchart.

8{1–6}, derived from 9a-aza-homoerythromycin-1-propylamine building block **4** and a set of isocyanate and isothiocyanate building blocks **5**{1–6} and **6**{1–6}, respectively (Chart 1), is shown in Scheme 1.

The synthesis of the amine **4** starts with the Michael addition of acrylnitrile on 9-deoxo-9-dihydro-9a-aza-9a-homoerythromycin A **9**. In the second step the cyanoethyl derivative **10** is hydrogenated with PtO_2 into the desired compound **4** (Scheme 2).[23] In this second step acetic acid was used as the solvent instead of ethanol. This modification allowed the reaction to finish in 12 h at 5 bar instead of 72 h at 20 bar, and additionally raised the yield of the product from 40% to 60%.

Initially, the chemistry sequence was developed and exemplified by the preparation of a representative 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) urea library **7**{1 and 6} and the corresponding 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) thiourea library **8**{1 and 6}. The transformation was optimized on an AdvancedChemtech PLS 4 × 6—synthesizer in 4 mL vials. Having developed the appropriate reaction conditions (Table 1), a set of small trial arrays ('rehearsals', Chart 1) were prepared to establish that each of the chosen building blocks performed acceptably under the reaction conditions chosen, before the full parallel library syntheses were initiated.

The preparation of the products **7**{1–6} and **8**{1–6} commences with the addition between an excess of the isocyanate or isothiocyanate (1.3 equiv) and the amine **4** to afford the desired urea derivatives **7**{1–6}, and the corresponding thiourea derivatives **8**{1–6} and was observed to proceed efficiently at ambient temperature in dichloromethane (CH_2Cl_2).

Following scavenging of excess **5**{1–6} and **6**{1–6} with a polystyrene-supported amino-resin (PS-trisamine, 4.11 mmol/g), the products **7**{1–6} and **8**{1–6} were isolated in good yields (73–90%) and high purity (90–96%; LC/MS). The use of a fivefold excess of the resin ensured that complete scavenging was achieved within 6 h. The removal of excess **5**{1–6} and **6**{1–6} could also be performed by the solid phase extraction methodology on a 1 g preloaded SiO_2 column.

However, we found that it is not possible to utilize a parallel synthesis strategy using aromatic and aliphatic ureas in the same library due to the big difference in the reactivity of the building blocks. The reaction time for the reaction with aromatic isocyanates was about 60 min whereas with aliphatic isocyanates it took

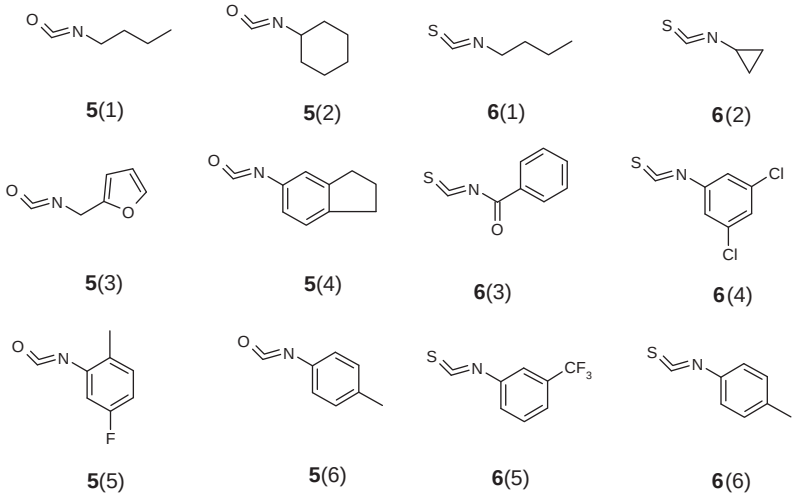
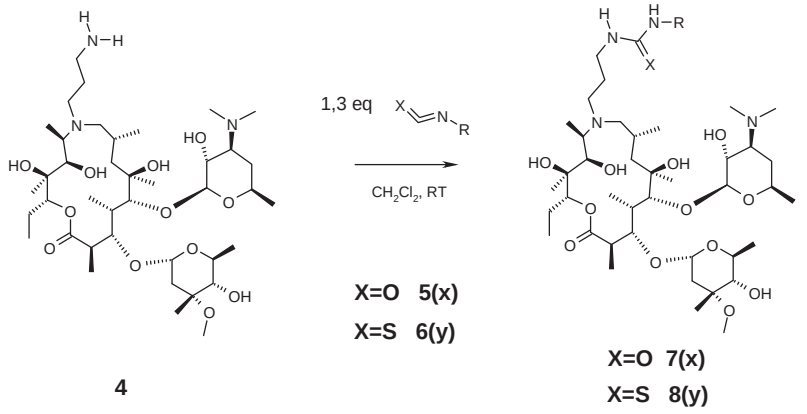
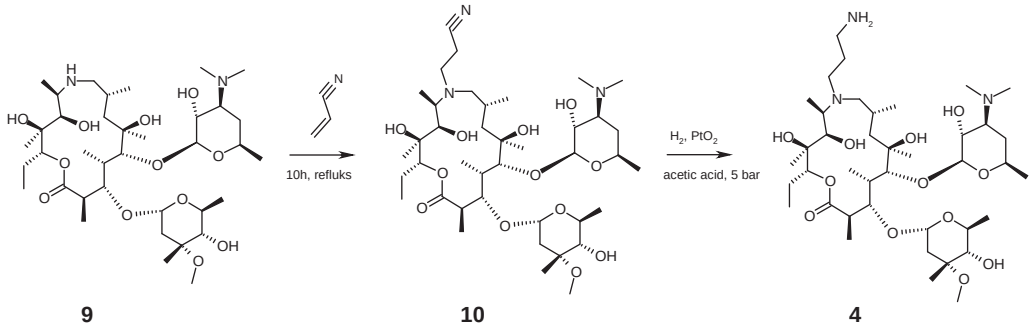


Chart 1. Set of isocyanate **5**(x) and isothiocyanate **6**(y) building blocks.



Scheme 1. Synthesis of urea and thiourea derivatives.



Scheme 2. Synthesis of 9a-*N*-(γ-aminopropyl)-9a-aza-homoerythromycin A **4**.

Table 1
Reaction conditions for urea- and thiourea libraries

X	R	Equivalents	Reaction time (h)
O	Aromatic	1.3	0.5–1.5
O	Aliphatic	1.3	12–24
S	Aromatic	1.3	12
S	Aliphatic	1.3	12–24

12–24 h. Such a big difference was not observed in the isothiocyanate series where the aromatic reagents reacted within 12 h and the aliphatic ones in 24 h (Table 1). Therefore, two separate libraries had to be prepared: one where aromatic ureas were synthesized and the second where aliphatic urea and thiourea derivatives were formed.

2.1.2. Building block rehearsal studies

Prior to committing to a full combinatorial library synthesis, it is advantageous to 'rehearse' the building blocks chosen in order to determine if they are compatible with and have sufficient reactivity's under the generic reaction conditions adopted. In this way, the potential for failure, or the isolation of large numbers of com-

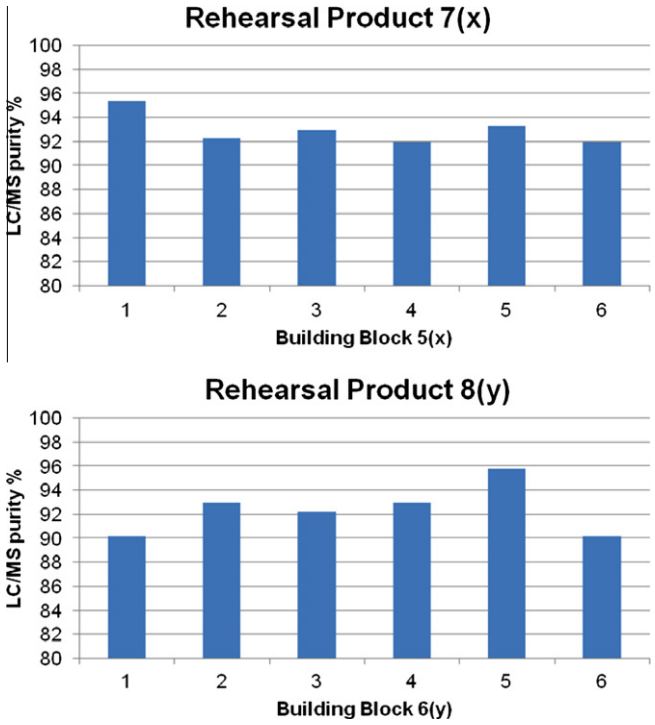


Figure 4. (a) Building block set **5**(x) rehearsal targeting product **7**(x). (b) Building block set **6**(y) rehearsal targeting product **8**(y).

pounds with low purities is reduced. The synthesis process of the urea and thiourea derivatives is described with the corresponding flowchart in Figure 3.

Using the previously determined conditions, the reaction between the amine **4** and the isocyanate and isothiocyanate building blocks **5**{1–6} and **6**{1–6} was performed in a 48-position Miniblock on the Mettler-Toledo MiniBlock synthesizer. A PS-trisamine scavenge was then performed to remove the slight excess of **5**{1–6} and **6**{1–6}, respectively that was used. In CH_2Cl_2 as the solvent, no insolubility difficulties were encountered and all urea derivatives **7**{1–6}, and the corresponding thiourea derivatives **8**{1–6} were isolated in 90–96% purity (LC/MS) (Fig. 4a and b). Although it is desirable for all products to have purities of >85%, a limited number of products with lower purities (60–85%), that may subsequently be purified by reverse-phase HPLC (RP-HPLC), are acceptable.

2.1.3. Preparation of a 33-member urea library 7{4–36}, and a 69-member urea and thiourea library 7{37–49} and 8{1–56}

The libraries were prepared using the reaction conditions developed for the synthesis of the trial compounds **7**{1–6} and **8**{1–6}. The process is outlined for the 33-member urea library derived from building block **4** and a set of aromatic isocyanate building blocks **5**{4–36} in Charts 1 and 2 and is the same as for the 69-member urea and thiourea library derived from building blocks **4** and **5**{37–49} and **6**{1–56} in Charts 1–3. Details of the programs used are given in Section 4.

Aliquots of the final product solutions were transferred and diluted to facilitate off-line LC/MS analysis of the library products. For the 33-member urea library **7**{4–36}, as shown in Figure 5a, 23 of the 33 library members (69.7%) were obtained in >85% purity (LC/MS) with an average library purity of 81.3%. For the 69-member urea and thiourea library **7**{37–49} and **8**{1–56}, 61 of the 69 desired compounds (88.4%) were obtained in >85% purity with an average library purity of 86.7%, according to LC/MS (Fig. 5a and b). In both cases, compounds falling below the 86% purity threshold, but with >60% LC/MS purities, were further purified by single-pass autopreparative RP-HPLC to afford additional samples for biological screening. The yield of both libraries was 69–95% overall, except for the compounds which had to be purified by RP-HPLC. Importantly, the potential for the loss of material through inefficient resin washing can be significantly reduced by the incorporation of thorough vortex mixing in the resin wash step. Even with these yields, however, we were still able to synthesize sufficient material for use in several biological assays.

Overall, full library analysis was accomplished with a randomly selected sample of 38 of the 105 compounds. This sample group was evaluated using ^{1}H NMR, ^{13}C NMR, LC/MS, and HRMS. Product purity was determined by LC/MS screening (ESI^+ detection)

2.2. Biological activity and SAR observations

The in vitro potency of 98 ureas and thioureas of 15-membered azalides was evaluated against three *P. falciparum* strains: TM91C235-multidrug resistant, D6-chloroquine sensitive and W2-chloroquine/pyremethamine resistant and compared to azithromycin in order to select compounds suitable for further testing. Parasites were exposed for 72 h. Data from in vitro potency tests of azithromycin against the three parasite strains were averaged, and these numbers were used to identify 'active' macrolide compounds. These average IC_{50}s against the TM91C235, D6, and W2 *P. falciparum* strains were 2065 nM, 1015 nM, and 2241 nM, respectively. Only compounds of equal or better potency than azithromycin against at least one of the parasites were selected for further consideration. Active compounds were sorted by IC_{50} against the TM91C235 clone (Table 2). Almost all 9a-N substituted 15-member azalide (91/98) showed better in vitro activity than azithromycin. In this assay, several compounds from the libraries were found to be very active against both resistant strains of the *P. falciparum*, with a notable decrease in parasitic growth. The most interesting compounds from this library, particularly in inhibiting the growth of all three strains including those that are highly drug resistant, were **8**{38}, **8**{37}, and **8**{25}.

The SAR observations commented here are primarily related to the TM91C235 results, but they are also in line with the observations for the other two strains. The influence of various 9a-N substituents were investigated using these synthesized compounds. Paired analogues differ only in the urea/thiourea moiety of the 9a-N substituent, and are presented in Figure 6. It seems that the thiourea moiety increases in vitro antimalarial activity in comparison to the urea moiety in most cases, with the exception of thiourea derivative that showed lower activity than the corresponding urea analogue [pair 11: **8**{55} < **7**{35}].

Generally, the in vitro activities of the compounds increase by increasing the rigidity of the 9a-N substituent making it energetically more favorable for possible intermolecular interaction. A simple descriptor—the ratio of rotatable bonds in 9a-N substituent—was used to describe the rigidity of 9a-N substituent. In vitro antimalarial activity increased as the ratio of rotatable bonds in the 9a-N substituent dropped (Fig. 7a). The average ratio dropped from 0.5 in inactive compounds ($pIC_{50} \leqslant 6$, $N = 10$) to 0.37 for highly active compounds ($pIC_{50} > 7$; $N = 22$). The ratio of rotatable bonds in 9a-N substituent was lowered mostly; (a) by increasing the number of aromatic atoms, and (b) by avoiding additional substitutions. Lipophilic 9a-N substituents (Fig. 7b) increased the activity of non-aromatic derivatives (e.g. in Fig. 8). However, these improvements were still significantly lower in comparison to the highly active aromatic 9a-N substituents.

Generally, an aromatic moiety in the 9a-N substituent improved the activity (Fig. 7c) compared to azithromycin—only one compound **5**(29) showed lower activity. Consequently, aromatic 9a-*N*

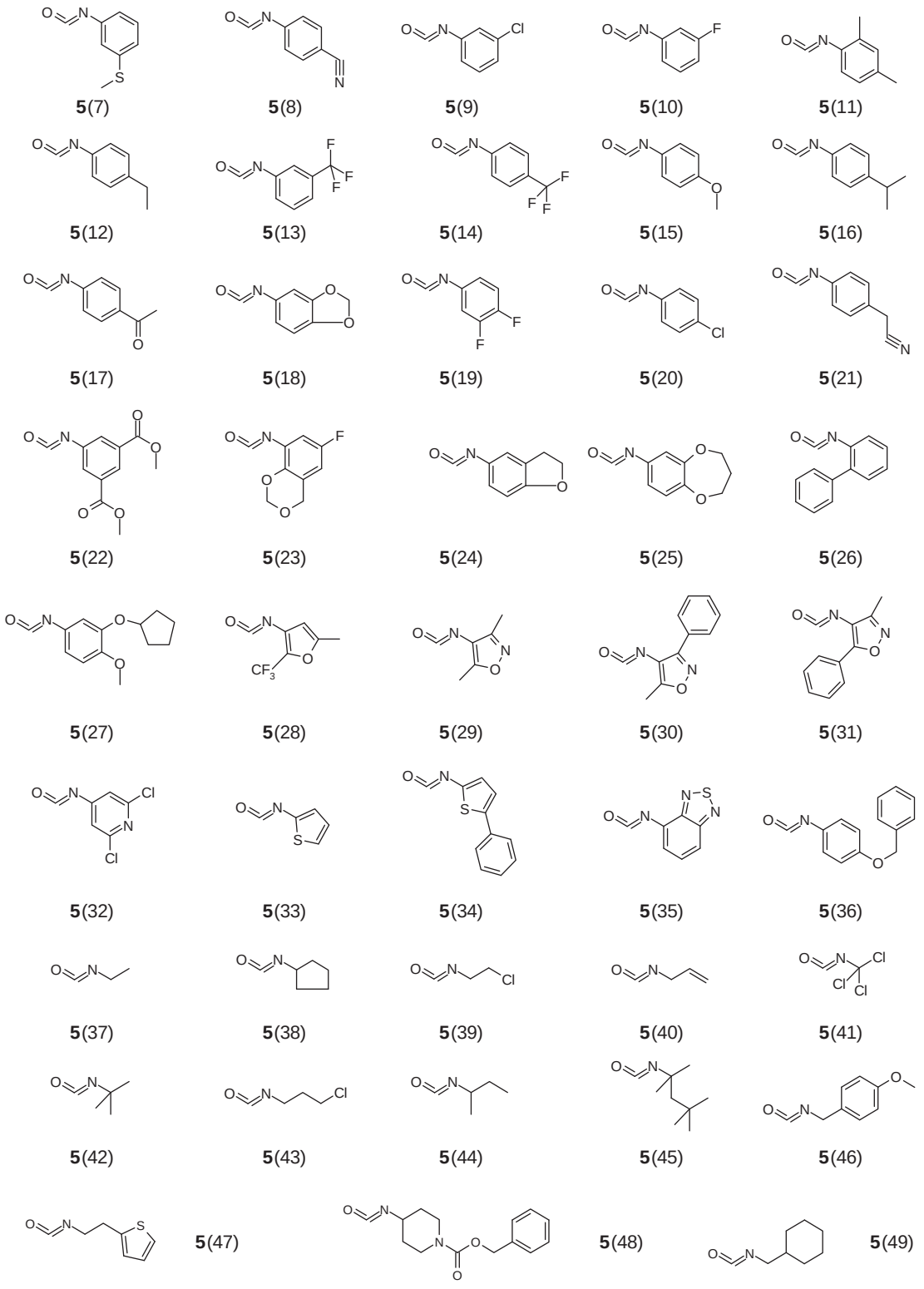


Chart 2. Set of isocyanate **5**(x) Building Blocks.

derivatives are preferred for further optimization in comparison to nonaromatic derivatives.

3. Conclusion

In conclusion, we have described an automated polymer-assisted solution phase protocol for the synthesis of 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) urea and 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) thiourea libraries. These methods have been exemplified by the preparation of a 33-member urea library **7**{4–36} and a 69-member urea and thiourea library **7**{37–49} and **8**{1–56}, using a Mettler-Toledo MiniBlock synthesizer. Building block rehearsals were performed in both cases in order to confirm that the monomers selected were likely to provide library compounds in acceptable yields and purities. In this way, ⩾48% of all library members were obtained with

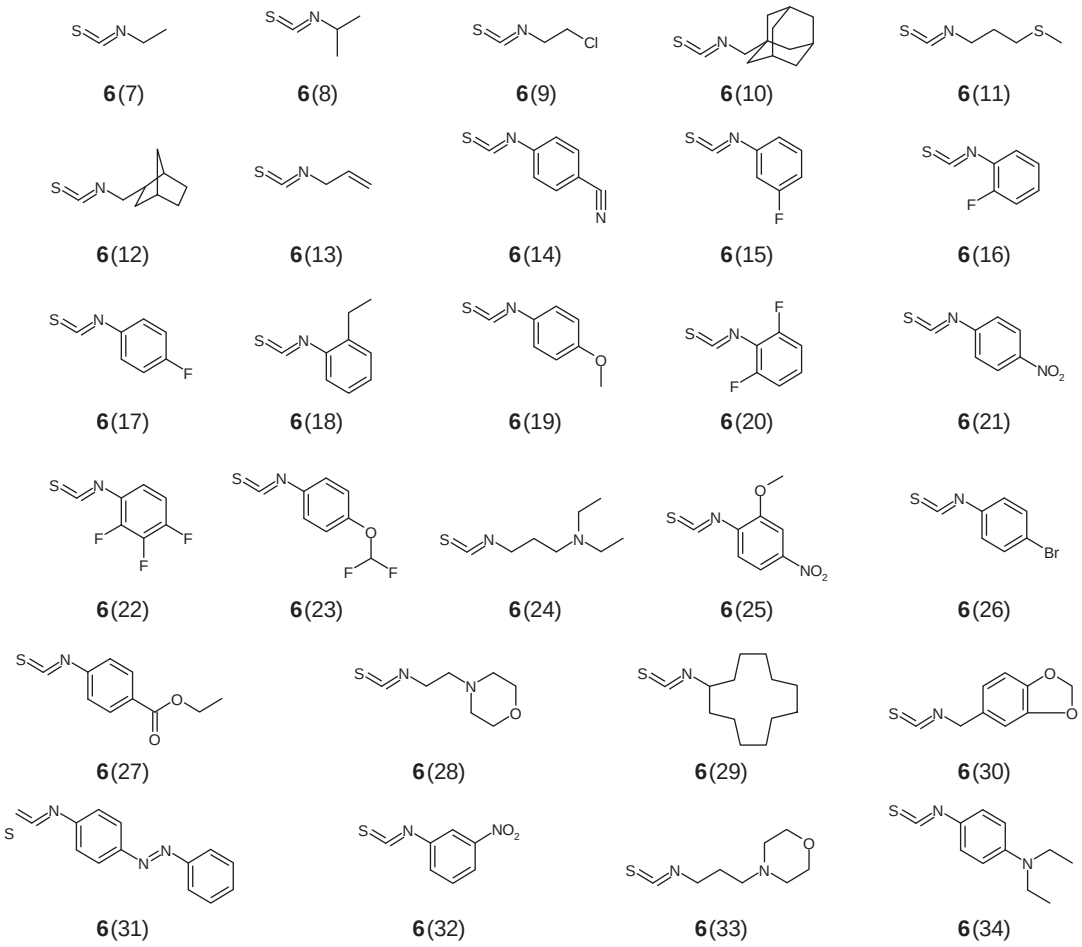


Chart 3. Set of isothiocyanate **6**(y) building blocks.

⩾90% LC/MS purities and these compounds required no additional purification prior to primary biological evaluation. The average purity of the raw products was 84.1%. In vitro potency data against three *P. falciparum* strains shows that almost all of the new analogues have greater potency than azithromycin, and the top 10 compounds are 30–65-fold more potent than azithromycin.

The present study confirmed the newly synthesized 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) ureas and 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) thioureas as new leads in antimalarial chemotherapy. Further optimizations regarding the functionality on the 9a-position as well as the in vivo profiling of the most interesting compounds are being envisaged as next steps. The outcome of these investigations will be shown in an upcoming publication.

4. Experimental section

4.1. General information

All solvents and reagents were used as supplied, unless noted otherwise. Isocyanates and isothiocyanates used in this work are commercially available chemicals and were obtained from Aldrich, Alfa Aesar, Lancaster and Maybridge. Polymer-supported reagents and scavenger resins were obtained from Argonaut Technologies.

Reaction preparation was carried out on a Mettler Toledo MiniMapper Sample Processing Station and the automated synthesis was performed using a Mettler Toledo MiniBlock system. Evaporation of solvents was conducted using a GeneVac HT-4X evaporation system. Analytical high-pressure liquid chromatography (HPLC) was carried out on a Waters FractionLynx LC/MS system using a Micromass ZQ mass spectrometer and positive electrospray ionization with a Waters Symmetry C8 4.6 mm × 75 mm, 3 μm; column. Preparative high-pressure liquid chromatography (HPLC) was carried out on a Waters FractionLynx LC/MS system using a Micromass ZQ mass spectrometer and positive electrospray ionization with a Waters Xterra RP18 19 mm × 100 mm, 5 μm; column.

HRMS data were acquired using a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 100–1100 Da, with a scan time of 0.9 s and an interscan delay of 0.1 s. Retention times (t_R) are reported in terms of minutes. NMR spectra were recorded in DMSO-d_6, using either a Bruker Avance III 600 (600 MHz) or DRX400 and an internal TMS standard. ^{1}H NMR spectra were recorded at 600 MHz or 400 MHz, ^{13}C NMR spectra were recorded at 101 MHz. All shifts are reported as part per million relative to TMS.

4.2. General automated procedure for the 48-membered library. Preparation of 9a-aza-homoerythromycin-9a-*N*-(γ-aminopropyl) urea and thiourea derivatives 7{4–36}, 7{37–49}, and 8{1–56}

Step 1. Weigh the building blocks and the amine needed for the reaction and calculate the solvent volumes to get the desired concentrations (0.1 mmol/ml—building blocks; 0.05 mmol/ml—amine).

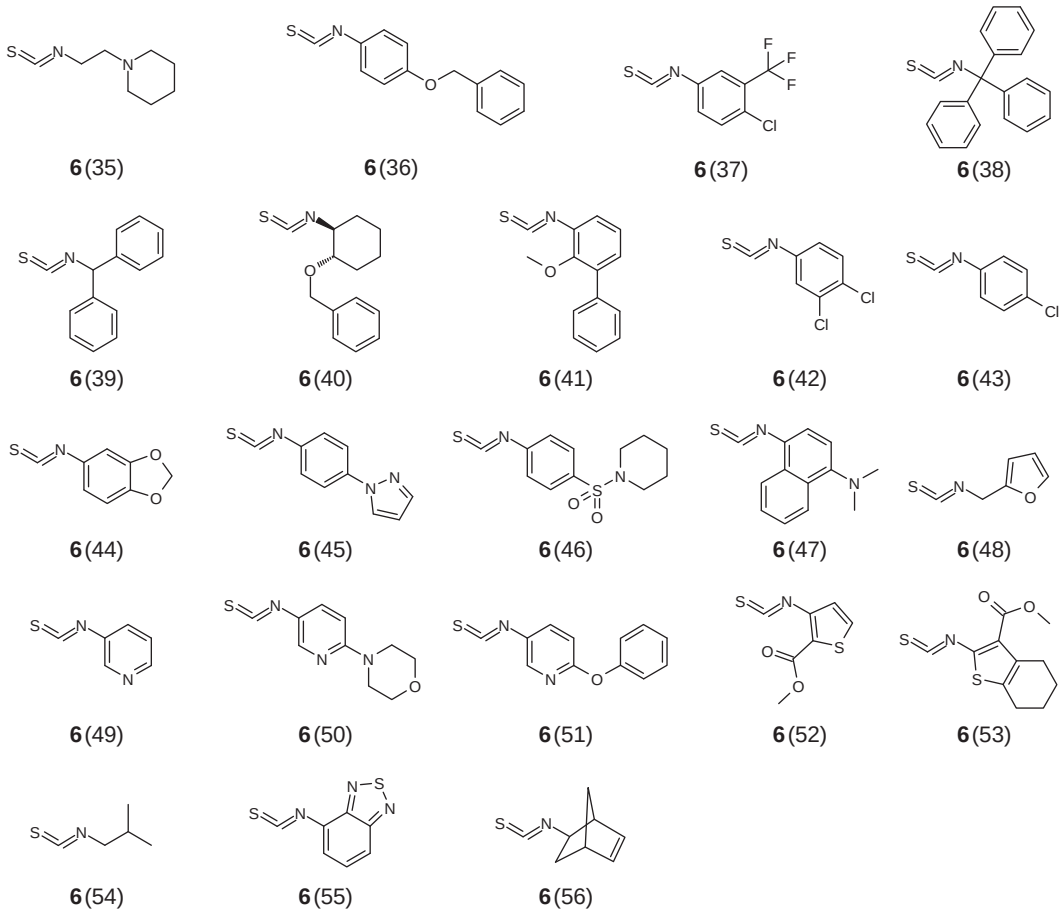


Chart 3. (*continued*)

Step 2. Put the vials with the building blocks and amine on the MiniMapper.
Step 3. Paste calculated volumes into the MiniMapper.
Step 4. Add the solvent (CH_2Cl_2) to the vials containing the building blocks.
Step 5. Add the solvent (CH_2Cl_2) to the 48-position reaction tubes of the MiniBlock synthesis module.
Step 6. Add the solvent (CH_2Cl_2) to the vials containing the amine.
Step 7. Transfer amine from the vials to the 48-position reaction tubes of the MiniBlock synthesis module.
Step 8. Transfer building blocks from the vials to the 48-position reaction tubes of the MiniBlock synthesis module.
Step 9. Remove reaction block from MiniMapper and place it on the MiniBlock synthesizer module and stir reactions vigorously at ambient temperature for 48 h.
Step 10. Add 5 equiv of PS-trisamine to every reaction tube and stir reaction at ambient temperature for 12 h.
Step 11. Drain resins and wash with CH_2Cl_2 (2 × 0.5 mL) into new vials.
Step 12. Prepare quality control (QC); dispense aliquots (5 μL) into 48 vials and dilute each vial with acetonitrile (100 μL) and water (900 μL).
Step 13. Remove solvent from bulk samples by parallel centrifugal evaporation in vacuum to afford the urea and thiourea derivatives.

Note: Compounds with crude purities <86% were purified by RP-HPLC.

4.3. General procedure for the parallel synthesis of urea and thioureas

To a solution of 9a-*N*-(γ-aminopropyl)-9a-aza-homoerythromycin A **10** (30 mg, 0.0378 mmol) in CH_2Cl_2 (1 ml), isocyanate or isothiocyanate (1.3 equiv) was added and stirred at room temperature for 2–48 h. At the end of the reaction PS-trisamine (5 equiv) was added to the reaction mixture. After stirring for 12 h at room temperature the resin was filtered off, and washed with CH_2Cl_2 (3 × 1 ml). The organic solvent was evaporated to yield the desired product.

4.3.1. 9a-*N*-(β-Cyanoethyl)-9a-aza-homoerythromycin A (10)

9-Deoxo-9-dihydro-9a-aza-9a-homoerythromycin A **9** (100 g; 136 mmol) was dissolved in acrylonitrile (300 ml) and the reaction mixture was heated at 80 °C for 24 h. The solvent was evaporated under reduced pressure to afford the desired product **10** (106 g) in 99% yield as a white foam. This compound was used for the next step without previous purification.

LC/MS (area %): 95%, MS(ES) *m*/*z*: [MH]+ 788.2 (calcd: 788.02).

4.3.2. 9a-*N*-(γ-Aminopropyl)-9a-aza-homoerythromycin A (4)

To a solution of 9a-*N*-(β-cyanoethyl)-9a-aza-homoerythromycin A **10** (5 g; 6.3 mmol) in glacial acetic acid (80 ml), PtO_2 (0.5 g) was added and the reaction mixture hydrogenated at a pressure of 5 bar for 12 h. The catalyst was filtered off. Water was added to the filtrate. The aqueous solution was washed with CH_2Cl_2 (3 × 30 ml). The pH of the aqueous phase was adjusted to 6, 8, 10, 12, and 14. At each pH the aqueous layer was extracted with CH_2Cl_2 (3 × 30 ml). The organic layers at pH 10 and 12 were com-

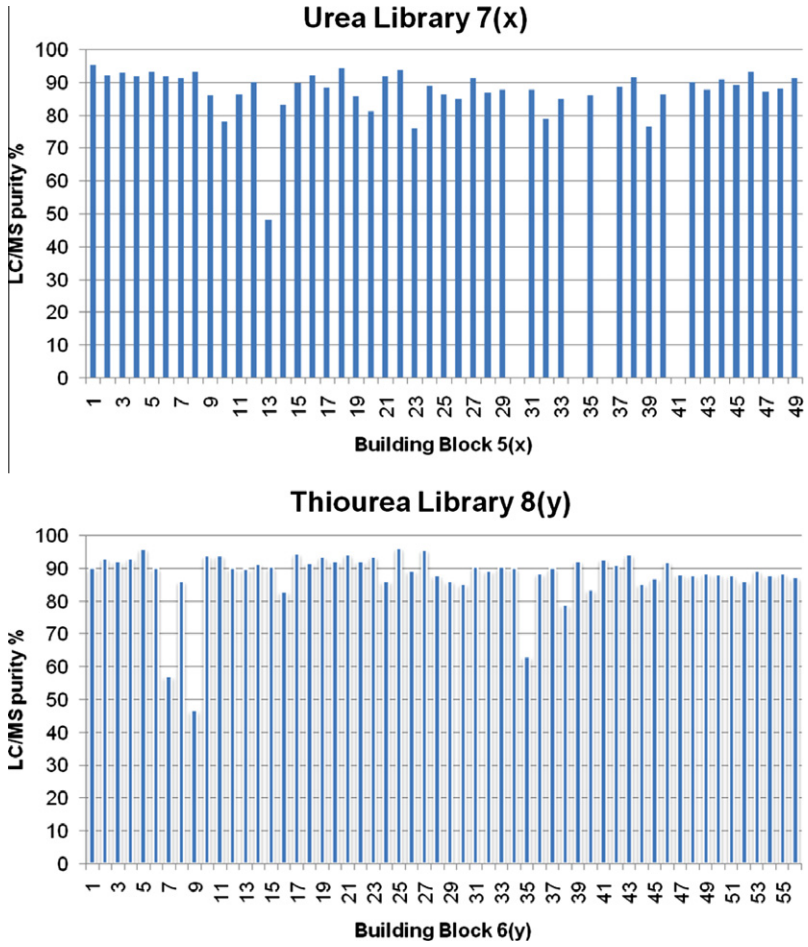


Figure 5. (a) Building block set **5**(x) library targeting product **7**(x). (b) Building block set **6**(y) library targeting product **8**(y).

bined, dried over Na_2SO_4 and the solvent evaporated under reduced pressure to obtain the desired product **4** (2.9 g) in 58% yield as a white foam.

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm: 0.77 (t, J = 7.33 Hz, 3H), 0.87 (d, J = 6.45 Hz, 3H), 0.94–0.98 (m, 6H), 0.99 (d, J = 7.33 Hz, 3H), 1.03–1.07 (m, 4H), 1.10 (d, J = 7.15 Hz, 3H), 1.12 (s, 3H), 1.14 (d, J = 6.11 Hz, 3H), 1.19 (s, 3H), 1.24–1.31 (m, 1H), 1.32–1.42 (m, 2H), 1.47–1.59 (m, 4H), 1.73–1.81 (m, 1H), 1.84–1.89 (m, 1H), 1.90–1.95 (m, 1H), 2.10 (t, J = 10.38 Hz, 1H), 2.21 (s, 6H), 2.27 (d, J = 15.00 Hz, 1H), 2.30–2.36 (m, 1H), 2.38–2.44 (m, 1H), 2.47–2.55 (m, 2H), 2.63–2.76 (m, 3H), 2.90 (d, J = 9.07 Hz, 1H), 2.95–3.00 (m, 1H), 3.01–3.05 (m, 1H), 3.21 (s, 3H), 3.48 (s, 1H), 3.50 (d, J = 6.63 Hz, 1H), 3.64 (dd, J = 10.03, 5.50 Hz, 1H), 3.99 (d, J = 5.93 Hz, 1H), 4.03–4.08 (m, 1H), 4.23 (br s, 1H), 4.40 (d, J = 7.15 Hz, 1H), 4.80 (d, J = 4.54 Hz, 1H), 4.90 (d, J = 10.47 Hz, 1H).

^{13}C NMR (101 MHz, DMSO-d_6) δ ppm: 7.24, 8.55, 9.97, 14.42, 16.83, 17.61, 20.01, 20.18, 20.55, 21.56, 26.77, 27.56, 29.38, 29.41, 34.00, 38.20, 39.56, 39.45, 39.50, 43.49, 47.90, 58.24, 63.07, 63.56, 63.97, 66.18, 69.82, 71.80, 72.67, 73.36, 74.13, 75.82, 76.48, 77.49, 82.71, 94.39, 101.16, 175.19.

LC/MS (area %): 89%. MS (ES) m/z: [MH]+ 792.3 (calcd: 792.05)

4.4. In vitro testing

The compounds were tested for their intrinsic antimalarial activity. The system is limited to the assessment of the intrinsic activity against the erythrocytic asexual lifecycle (blood schizontocides). Two Plasmodium falciparum clones from CDC/Indochina III (W-2) and CDC/Sierra Leone I (D-6) were used for all assays. TM91C235, a multiple drug resistant isolate from Thailand, was used for the prescreening assay. W-2 is resistant to chloroquine, quinine, and pyrimethamine and susceptible to mefloquine. D-6 tends to be more resistant to mefloquine and susceptible to chloroquine, quinine, and pyrimethamine. The isolate was preexposed, in duplicate, at three concentrations (25,000 ng/ml, 2500 ng/ml, and 25 ng/ml) of the test compound for 72 h in a 96-well microtiter plate (MTP) using the BIOMEK® 2000 automated laboratory workstation. The assay relies on the incorporation of radiolabeled hypoxanthine by the parasites, and inhibition of isotope incorporation is attributed to activity of known or candidate antimalarial drugs. After 96 h of total incubation time, the MTP were frozen to lyse the erythrocytes and parasites. The parasite DNA was recovered by harvesting the lysate onto glass-fiber filter plates using a Packard FilterMate Cell Harvester. The radioactivity was counted on a Packard TopCount microplate scintillation counter. The results were recorded as counts per minutes (CPM) per well at each drug concentration divided by the arithmetic mean of the CPM from the three untreated infection parasite control wells.

4.5. SAR analysis

SAR analysis was performed using *D-Score* and *Find Lookalike* procedures implemented as a part of the SAR Toolkit,[24] a GSK

Table 2
In vitro activity of tested compounds against three *P. falciparum* strains with different susceptibility patterns

IC_{50} (nM)			Compound
Multidrug resistant TM91C235	Chloroquine sensitive D6	Chloroquine/pyrimethamine resistant W2	
42.4	5.6	166.6	Chloroquine
2065	1015	2241	Azithromycin
31.9	124.8	37	8{38}
42.9	53.4	48.3	8{32}
51.3	102.8	37.1	8{21}
54	119.8	30.3	8{5}
55	218.3	37.7	8{39}
55.3	58.1	113.5	8{41}
55.4	115.7	42.5	8{4}
56.3	54.3	35	8{37}
60	28.1	40.8	7{25}
64.7	266.5	69.2	7{35}
68	174.5	53	8{46}
69.7	74.5	50.1	8{25}
72.8	256.6	134.9	7{13}
73.7	449.2	164.7	8{22}
78.2	132.2	50	8{14}
78.3	224.6	70.9	7{16}
84.2	159.9	74.6	7{14}
84.2	296.2	229.4	8{42}
90.4	188.1	99.3	7{20}
96.2	265.5	73.6	7{4}
96.3	107.4	47.9	8{31}
99.3	226.1	73.8	7{12}
101.2	160	109.4	8{45}
101.4	175.8	88.2	8{55}
101.8	248.9	46.5	8{12}
106.7	121.3	70.9	8{27}
107.5	187.7	53.4	8{43}
115.6	511.2	199.8	8{15}
116.9	396	53.2	7{5}
117.7	454.9	187.2	7{9}
121.1	216.4	63	8{23}
122.5	986.7	399.6	8{16}
126.5	644.8	273.8	8{40}
127.3	299.6	89.6	7{19}
132.7	215.3	109.2	8{51}
137.1	332.4	73.9	7{7}
137.1	253.6	202.1	7{26}
138.1	445.2	201.4	7{27}
138.6	319.8	72.8	8{17}
138.8	330.1	88.7	7{6}
140.1	264.5	85	8{29}
142.4	609.5	113.1	7{49}
146	298.6	175	7{36}
152.2	552.8	210	8{10}
159.5	249.3	88.9	8{36}
163.8	579.7	285	7{10}
164.1	330	71.3	8{6}
172.9	329.4	182.5	8{44}
173.3	260.8	168.9	8{26}
179.9	326	174.9	8{47}
182.9	302.1	172.6	8{56}
183	268.6	78.4	7{45}
188.4	508.7	86.3	8{20}
206.2	386.2	98.3	7{8}
209.4	480.4	77	8{19}
216.5	319	136.6	8{30}
219.8	283.9	306.1	7{23}
232.3	469.8	178.6	7{11}
236.2	635.1	276.2	7{48}
239.9	329.4	448.9	8{48}
251.7	444.9	96.2	8{18}
265.4	835.2	110.9	7{18}
288.2	297.5	107	8{3}
298.3	765.3	683.9	8{34}
320.4	360.6	309.5	7{28}
330.4	452.5	374.7	8{52}
353.8	897.8	202.3	8{11}
381.5	655.2	187.8	8{1}

Table 2 (*continued*)

IC_{50} (nM)			Compound
Multidrug resistant TM91C235	Chloroquine sensitive D6	Chloroquine/pyrimethamine resistant W2	
398	1730	708.9	7{17}
405.1	931.7	1043.1	7{47}
407.1	824.7	229.3	8{53}
410.8	647.6	119.8	7{22}
411	1961.2	184.7	7{2}
428.5	2605.1	1298.1	8{8}
434.6	688.9	495.1	8{54}
441.5	1965.4	255.8	7{15}
483.9	1620.2	203.8	7{46}
491.7	936.2	734.3	7{31}
495.5	5614.4	287	7{3}
514.5	1141.1	276.1	8{13}
548.7	964	720.6	7{33}
570.9	1788.3	148.7	7{21}
615.6	6022.4	277.3	7{38}
799.7	5505.6	410.5	7{42}
801	1211	1198	8{49}
820.2	5802.2	370.2	7{1}
923.3	2051.4	1585.3	7{24}
971.6	3721.9	679.1	8{2}
1175.5	14025.9	488.6	7{44}
1569.1	2842.1	1957.6	8{50}
1980.7	13748.7	13748.7	7{37}
3028.9	9286.4	3297.9	8{35}
3319.1	12372	2090.7	8{28}
5091.1	7008.5	1273.1	8{33}
12371.4	794.2	3132.5	8{24}
12804.4	12804.4	12804.4	7{29}
12837.4	12837.4	12837.4	8{9}

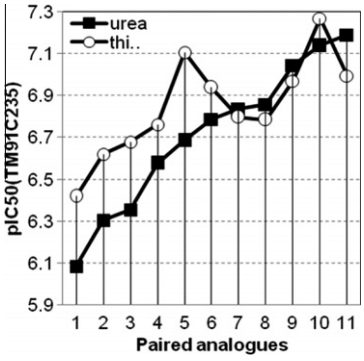


Figure 6. In vitro antimalarial activity against *Plasmodium falciparum* TM91C235 strain of paired analogues of 9a-N substituted 15-membered azalides differing only in urea/thiourea moiety.

proprietary suite of applications for SAR data analysis, based on Daylight Toolkit[25] and embedded within Spotfire DecisionSite.[26] *D-Score* is a method for the identification of molecular pairs differing by a single R-group change upon fragmentation of the analyzed molecules. D-Score was calculated simply by averaging the difference in activity between two paired molecules over the number of pairs. Pairs of molecules which differ by a single R-group change were extracted and visualized using profile plots; it can be useful to identify basic SAR trends as well as outliers, to investigate additivity in the data, and potentially predict novel combinations. *Find Lookalike* is a method for the selection and visualization of molecules that differ by a single localized change for the investigation of basic SAR trends. This structural change may involve one, two or up to a user-defined number of atoms as long as they are connected. Any change in activity/property is clearly related to the single change in structure.

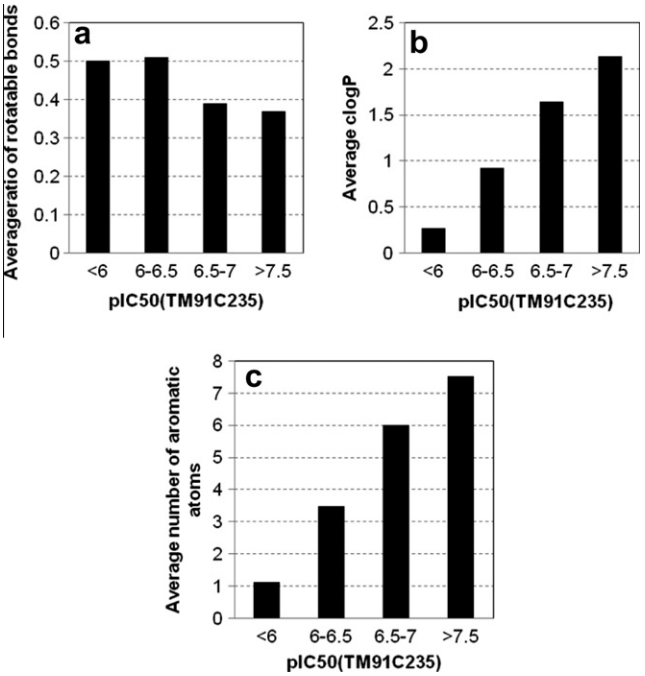


Figure 7. In vitro antimalarial activity against TM91C235 *Plasmodium falciparum* strain for 15-member azalides increases with (a) decrease in the ratio of rotatable bonds in 9a-N substituents, (b) increase in lipophilicity of 9a-N substituents; and (c) with increase in the number of aromatic atoms in 9a-N substituent.

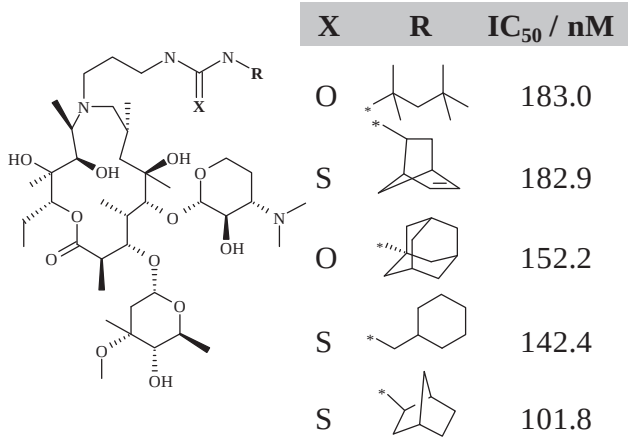


Figure 8. Nonaromatic lipophilic 9a-N substituents increased in vitro antimalarial activity against TM91C235 *Plasmodium falciparum* strain in 15-member azalides.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.030.

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